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The Cytology of the Pituitary Gland of Two Varieties of Goldfish (*Carassius auratus* L.), with Some Reference to Variable Factors in the Gland Which May Possibly Be Related to the Different Morphological Types.

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(Plate I).

In recent years the pituitary gland, as compared to other glands of internal secretion, has received a greater amount of concentrated attention and experimental study. However, most of this work has centered around the pituitary of the mammal and only a few investigators have attempted to give any sustained attention to that structure in lower forms. This seems rather unfortunate for the greater part of the confusion, regarding the pituitary, concerns itself with its supposed manifold hormonal activities and inter-relationships. In the higher forms, for example, such functions as corpus luteum formation, uterine growth, lactation and proportional growth in general, have all been shown to be related to the pituitary gland. In lower forms most of these physiological activities are absent. This alone should result in a comparative simplification of the functional activity of the gland. Therefore, it is reasonable to believe that a study of the pituitary gland in the lower forms may lead more readily to a better understanding of the function of the gland. However, before the function of any gland may be adequately studied, it is always valuable to have as complete a knowledge of its microscopic structure as possible. This, of course, becomes especially true if any attempt is made to correlate its function with its structure. In addition, there is still a great need for a cytological study of the pituitary in these lower forms by the use of modern techniques (Charipper, 1937).

The morphology and microscopic anatomy of the pituitary gland of only a few species of Cyclostomes, Pisces and Amphibia have been worked out with varying degrees of completeness. This work has been covered for the most part by Tilney (1911), Stendell (1914) and Bock (1928). More recently, Bell (1938) has reviewed the previous work on the pituitary of the lower vertebrates and has added a morphological description of the pituitary gland of the common goldfish (*Carassius auratus*). None of these workers has attempted a complete cytological description of the cells forming the gland in the species which they considered.

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[1] Accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, New York University.

Aside from the work on amphibian metamorphosis, there has been very little, if any, investigation of the relationship of the pituitary in lower forms to deviations from the normal body structure known to occur in such animals. Cushing (1932, a, b) and his co-workers have described cellular changes in the anterior lobe occurring in acromegalic individuals and those suffering from "Basophilia." Keith (1922), Crew (1923) and Stockard (1936) have also related body abnormalities to changes in the endocrine glands. These workers believe that these structural abnormalities are carried on from generation to generation.

The present work has two objectives: (1) to describe the histology and cytology of the pituitary of two varieties of goldfish, and (2) to investigate whether or not easily apparent differences in body structure occur along with variations in the pituitary.

I wish to acknowledge gratefully the interest and helpful suggestions of Dr. Harry A. Charipper under whose guidance this work was carried out. I wish also to thank Dr. C. M. Breder, Jr., of the New York Aquarium for his invaluable assistance and co-operation.

Materials and Methods.

The pituitary glands of two varieties of goldfish (*Carassius auratus* L.) were examined both histologically and cytologically. They were removed from animals, both male and female, purchased at local supply houses during the months from October to March. The varieties of goldfish studied were the "common" and the "telescope moor." The former has an elongate body, a scaleless head, yellow to gold pigmentation and two sets of paired fins, the ventral and pectoral, as well as three single fins including the tail. The "telescope moor" has a short blunt body which is rounded and egg-shaped and possesses a black pigmentation. All the ventral fins are paired and its tail is divided to its base. In this variety the eyes are set into stalks which project from the sides of the head.

To follow cytological procedures, it was found best to remove the pituitary gland from the animal before placing it into the fixing solutions. In order to obtain the gland, the animal first was quieted in an ice water bath. The head then was severed from the body, the ventral surface of the brain case exposed and the portion of bone below the gland carefully cut away. The exposed gland was removed easily and placed into the fixing solution.

The majority of the pituitary glands were fixed, either by the method of Champy or by Nassonov's modification of the method of Kolatchev. In addition, a few of the glands were fixed according to the Mann-Kopsch method as described by Gatenby. Pituitary glands were also treated according to the Champy-Kull method in order to demonstrate mitochondria.

After fixation and impregnation, all the glands were dehydrated and cleared in dioxan. Dioxan was advantageous in keeping the brittle osmicated tissue from fragmenting. Sections were cut in hard paraffin (60° C.) at two, three and four micra at low temperatures. Glands were sectioned serially in frontal, sagittal and horizontal planes, and stained by either the Masson method or the Dawson modification of the Heidenhain azan procedure.

It is important to note that throughout the work the pituitary glands of the two varieties of goldfish being studied were fixed in sets and run through all the procedures side by side thereby assuring the validity of cytological comparison. Further, the glands were removed from animals of approximately the same weight.

Observations and Description.

The pituitary gland of the goldfish (*Carassius auratus*) lies below the brain and is connected with it by a short heavy stalk. A tough membrane separates the ventral surface of the brain from the pituitary gland. The infundibular stalk leaves the brain case, along with the optic nerves, through a foramen in the parasphenoid bone. After emerging from the foramen, the stalk continues posteriorly as the pars nervosa and forms a center about which the rest of the gland is arranged. Part of the pituitary gland also extends anteriorly and, as the gland is applied closely to the ventral surface of the brain case, it acts to close the large optic-pituitary foramen. These morphological relationships confirm the findings of Bell (1938).

The pituitary glands of two varieties of goldfish, the common and the moor, were examined cytologically. No easily apparent differences were found in cell types, cell structures or Golgi configurations. Therefore, the following description of the gland may be applied to the pituitary of either the common or black moor varieties.

The pituitary gland is divided, as Bell (1938) has shown, into four easily distinguishable areas. The infundibular stalk enters the gland and is continuous with the pars nervosa which ramifies throughout all parts of the gland. The blood supply of the gland is intimately connected with these ramifications (Plate I, Fig. 9). Blood capillaries are only present between the fibers of the nervosa tissue. The cells found among the pars nervosa fibers are few in number.

Pars intermedia.

The entire posterior end of the goldfish pituitary gland consists of a compact intermediate lobe. The pars nervosa ends here, after having passed through the übergangsteil or transitional lobe, as a heavy clump of fibers which spread out in all directions. The cells of the pars intermedia are arranged along the invading pars nervosa and are large, measuring from ten to fifteen micra in diameter. The cells composing the entire free surface of the intermediate lobe contain many osmiophilic granules while in the interior of the lobe and along the attached border there is a mixture of granular and non-granular cells (Plate I, Fig. 1). These are oriented along the strands of invading nervous tissue. Only granular cells, however, are found in the spaces between the strands of nervosa tissue. The non-granular cells are vesicular, elongate, and contain large nuclei which are clear after Champy fixation. The granular cells are larger and contain nuclei which, after the same fixation, stain a solid red with acid fuchsin. Even after ordinary fixation, the nuclei of the two types of cells can be distinguished by a difference in intensity of staining. They both contain fine strands of nuclear material in a clear nucleoplasm. These nuclei are round or oval after most types of fixation, but take on many bizarre shapes and stain deeply red after exposure to an osmo-sublimate solution.

The cytoplasm of the intermedia cells does not stain in a similar manner following the use of different fixing solutions. After Bouin fixation, a blue granulation is present, while following osmic acid fixing solutions the cytoplasm stains light blue or not at all.

After exposing the intermediate lobe cells to osmic acid fumes, a Golgi apparatus appears in these cells (Plate I, Fig. 2). It is of the same type and occupies the same position in both the granular and non-granular cells. The osmiophilic substance forms a circular cap which is applied closely to the nucleus and never extends over more than one-half of its surface. On cross section it is usually granular although in some cells several strandlike layers may be found. Sometimes, tiny vesicles are seen between the strands. After the same length of exposure to osmic acid the granular cells have a much blacker and more extensive Golgi apparatus than the non-granular cells. There is no apparent orientation of the Golgi material in relationship to the position of the cell. In one cell the network may be found applied to the nucleus on the side nearest the invading nervosa tissue while the next cell may have its Golgi network on the opposite side of the nucleus.

After the use of the Champy-Kull technique and staining with acid fuchsin, the mitochondria, which are in the cytoplasm on one side of the nucleus, appear as fine granules. In some cells the granules spread out to fill up more than one-half of the cell cytoplasm.

Pars anterior.

Each of the cells along the outer surface of the anterior lobe contains a small nucleus, placed at one end of a clear cytoplasm. These cells are from six to ten micra in diameter and are grouped closely together (Plate I, Fig. 8). Their nuclei, after Champy fixation, are clear, while after non-osmic acid fixing solutions they contain a great many chromatin strands and granules. Nucleoli which stain heavily are present at one edge or in the center of the nuclei. The cytoplasm surrounding the nuclei is filled with a flocculated non-stainable material. A few osmiophilic granules are found near the nuclei and a Golgi apparatus is present. This Golgi figure is small, granular, and applied closely to the surface of the nucleus (Plate I, Fig. 3). It covers an area about one-fourth that of the nucleus, and is situated on the side which contains the most cytoplasm. No radiating strands or granules leave the loosely collected central portion of the apparatus to pass into the surrounding cytoplasm. The osmiophilic granules, even after prolonged exposure to osmic acid fumes, do not become greatly blackened. There is no orientation of the Golgi material in respect to the pars nervosa which spreads throughout the lobe.

In addition to the cells which have been described above, there are others which are smaller in size and fewer in number. These cells contain small nuclei which stain similarly to those found in the non-granular cells. The cytoplasm of these cells stains a reddish brown. It contains two types of granules, one of which stains with acid fuchsin and the other which becomes blackened after exposure to osmic acid. These granules fill the entire cell and completely surround the nucleus. A Golgi network is next to the nucleus and is of the same size and extent as that found in the non-granular cell. However, the apparatus is strand-like and contains an increased amount of osmiophilic material which results in more intense blackening. Along the anterior surface of the lobe these cells are few in number and lie between the non-granular cells while in the deeper parts of the lobe large groups are found. They are most numerous at the border between the anterior and transitional lobe.

A third variety of cell is present in the anterior lobe. It is as large as the non-granular cell and has a cytoplasm which contains a few granules. The nucleus is similar to that present in the other types of cells found in this lobe, and is at the edge of the cell. In some of these cells the cytoplasm, immediately surrounding the nucleus, is clear while that at the outer rim is highly granular. In others, the clear area of cytoplasm has increased and only a few granules remain. Cells are present which show different degrees of granulation, ranging from a completely granulated cytoplasm to one containing no granules at all. Thus, these cells may be intermediate cell stages between the clear cells found along the periphery of the lobe and the highly granular cells found in the interior of the lobe. These cells are found mainly at a point midway between the inner and outer edges of the anterior lobe, where the lobe extends deeply into the transitional lobe.

The Golgi apparatus in this third variety of cell is similar to that found in both the granular and agranular cells. It is not as strand-like and heavily staining as the one in the granular cells but is less granular and stains more deeply than does the apparatus in the non-granular cells. The appearance of Golgi apparatus and the changes in granulation from a granular to a non-granular cell seem to indicate a morphological relationship between all the cells described.

Occasionally a basophile is found among the acidophiles of the anterior lobe. This cell resembles in form and structure the basophilic cells which are present in the transitional lobe. It is larger than the acidophiles, has a smaller nucleus and a cytoplasm which may be highly granular or have a homogeneous structure. The blue-staining granules, when present, are very large and few in number, and are found in a background of more finely granulated material which take a light blue stain. The basophiles generally appear singly, although a group of four or five is seen occasionally. In each cell a heavy strand Golgi network radiates from the nucleus. It is not as small or compact as that present in the acidophiles.

Transitional lobe.

A general survey of the transitional lobe of the goldfish pituitary gland is somewhat confusing in that there does not appear to be any definite architectural pattern, such as a nesting or cordlike arrangement, present anywhere in this region of the gland (Plate I, Fig. 4). The fibers of the nervosa ramify, apparently at random, passing as readily between groups of basophilic staining cells as between acidophilic staining cells. Occasionally, bands of nervosa fibers completely surround a group of cells, tending to separate them from their neighbors. The cells in such groups do not appear oriented in any definite way with respect to the investing fibers. It seems that either chromophobes, acidophiles, or basophiles may be opposed to the nervosa tissue.

Histological examination of carefully prepared sections of the gland, in which the transitional area has been treated according to the method of Severinghaus, discloses many different cellular entities. These different cell types can be distinguished, one from the other, by their variation in size, granular or agranular cytoplasmic nature, and chromophilic reactions. The following three major types of cells are found in this portion of the pituitary:

(A) The granular basophilic cells which are characterized by the presence of many large, spheroidal deep blue staining cytoplasmic bodies set in a gray matrix, and crowding on to a large vesicular nucleus.

(B) The granular acidophilic cells which are somewhat smaller than the preceding type and diagnostically contain a fine eosinophilic granulation set in a light clear cytoplasm and surrounding a large vesicular nucleus.

(C) The chromophobic cells which are smaller than any of the other cell types and contain large clear vesicular nuclei set in a scant, non-staining, agranular cytoplasm.

It is interesting to note that both the granular basophilic cell type and the granular acidophilic cell type may be found in an agranular form. Accompanying the degranulation of these types, the nucleus undergoes a change from the large clear vesicular karyosome to a compact almost pycnotic one.

A careful study of the transitional region, in frontal and sagittal serial sections, discloses a rather characteristic distribution of the various cell types in relation to one another. The acidophiles and basophiles are present throughout the lobe as aggregates, among which may be found a few chromophobes (Plate I, Fig. 4). The acidophiles vary in number in different parts of the lobe. They are relatively more numerous in a mid-sagittal section than in a section near the periphery. The basophiles are found in greater numbers along the dorsal border of the lobe, posterior to the overlying pars anterior, and also appear to be more compactly grouped at the antero-ventral border, posterior to the stalk as it leaves the body of the gland. In addition, a large group of basophiles border the transitional lobe in the area of its contact with the pars intermedia. The chromophobes are the least numerous of the cell types found in the transitional lobe. These cells occur scattered in groups of two and three, throughout the lobe, among both the basophiles and acidophiles. Small groups of chromophobes are present along the nervosa tissue which extends between the transitional and intermediate lobes. Much larger groups are to be found associated with the nervosa tissue passing through the substance of the transitional lobe. In fact, in frontal section, the chromophobes are seen to surround completely the larger branches of the nervosa tissue.

A close study of the individuality of these various cell types under conditions of special techniques presents some interesting configurations of the Golgi material and mitochondrial substance. Both Golgi nets and granular mitochondria are demonstrable in all the cell types to be found in the transitional lobe. The Golgi material, however, presents various differences in configuration which are characteristic of the different cell types. In the acidophiles this osmiophilic substance is compact and closely applied, in the form of a cap, to the side of the nucleus (Plate I, Fig. 5). It consists of several heavy strands which form short loops in the cytoplasm. These loops do not extend very far from the nucleus and free ends from the network are rarely seen. In some instances, these cells contain a larger Golgi net which extends a considerable way out into the cytoplasm. The mitochondria in these cells are present throughout the cytoplasm, in the form of a fine granulation. Areas of condensation may be present on one side of the nucleus in the region of the Golgi net. The mitochondrial granules can easily be differentiated from the secretory granules, after proper destaining, by their lighter pink coloration.

The Golgi configuration present in the basophile is more strand-like and more extensive than that found in the acidophilic cells (Plate I, Fig. 7). Here again the Golgi material is limited to one side of the nucleus, but does not form a cap. Instead it appears to be free in the cytoplasm only occasionally touching the nucleus and then only at a point or two. The loops are large and made up of fine strands of osmiophilic material. The cytoplasm between the strands is finely granular and the large basophilic granules or spheroids are found completely outside the region of the net and along the borders of the cell. The mitochondria here are also granular and are dispersed throughout the cytoplasm. The finer granular chondriosomes are found nearest the nucleus while the coarser ones are toward the periphery and in between the basophilic secretory granulations. There does not appear to be any points of increased condensations.

The chromophobes present a series of extremely interesting Golgi configurations (Plate I, Fig. 6). All of these cells have a characteristic compact cap of osmiophilic substance which is limited to one side of the nucleus. This Golgi material forms either a high or low triangle. The high triangle Golgi network consists of fine strands of osmiophilic substance and appears to tend more toward fine loop formation while the low triangular form is made up of coarser strands, compactly arranged with only an occasional visible opening. Finely granular mitochondria are present in both these varieties of cells and are found to be unevenly dispersed in the narrow rim of cytoplasm surrounding the nucleus.

Cell Counts.

In the absence of any obvious difference between the two varieties of animals studied, it was deemed advisable to resort to the method of differential cell counts. This method should bring to light such differences in the numerical distribution of cell types which may be present and yet not be apparent under routine microscopical examination. Initial samplings of cell counts of the transitional lobes of many glands, chosen at random, were made first. Such studies yielded results which warranted a further and more careful examination of the percentages of chromophilic cells in the glands of both varieties. The transitional lobe was chosen for examination because it contained the different varieties of chromophiles in greater number.

Differential cell counts were made on the chromophilic cells of the transitional lobe of the pituitary glands of both varieties of goldfish. The animals compared were similar in weight and since they were purchased from local supply houses their ages could only be approximated. Pituitary glands removed from both sexes of each variety were studied. Care was taken to compare the glands which had been fixed and stained simultaneously, thereby eliminating any possible errors due to differences in techniques. The results of these cell counts showed that the transitional lobe of the pituitary gland in both male and female animals of the same variety, contained similar percentages of basophilic and acidophilic cells, thus indicating that the sex of the animal could be only, at best, a slightly modifying factor. The pituitary glands were removed from all the animals during a normally occurring sexually inactive period.

With the aid of an ocular counting chamber comparable areas were selected and the chromophilic cells in one hundred squares of the chamber counted. The following precautions were taken to insure a normal sampling and to minimize the possibility of selecting areas subjectively: Firstly, using low power magnification, the anterior end of the gland was always placed against the highest horizontal line of the counting chamber and the ventral surface of the gland oriented against the most lateral vertical line. Secondly, after the section to be studied had been oriented under low power, the oil immersion objective was placed in position and the cells falling within the one hundred squares were counted. Depending upon the thickness of the serial section, every third or fourth section was examined. The cell counts throughout the lobe were made at intervals of twelve micra. An average of 1,000 cells were counted from each gland. Table I gives the results of such counts.

A statistical method was used to evaluate the results obtained. Although many pituitaries were studied, only those in which every section of the gland was properly cut, mounted, and stained were used for calculation and analysis. The validity of the statistical results rests, in part, upon the perfectness and completeness of the sets of serial sections. In view of the extremely small coefficient of variation (see Table I) obtained in this work and the fact that the statistical method employed in this analysis permits the use of few determinations, seven different animals of each variety were employed. In addition, the rigid criteria of technique applied in the choice of the series used, made it expedient, without detracting from the validity of the analysis, not to increase the number of animals.

Discussion.

The pituitary glands of the two varieties of goldfish studied in the present investigation are similar in their anatomical relationships and gross morphological subdivisions. These findings confirm the description which Bell (1938) gives for the common variety. The four different lobes described for the goldfish are easily distinguished one from the other by their anatomical position and by their reactions to polychromatic stains. The presence of a pars nervosa, pars intermedia, pars anterior and a special portion referred to as the übergangsteil or transitional region is typical of the pituitary in all forms of fish thus far studied (Stendell, 1914; Bock, 1928; Matthews, 1936; and Bell, 1938).

The blood supply of the pituitary gland is intimately connected with the pars nervosa and no sizable vessels are found entering the gland from its surface. A similar condition has been reported by Stendell (1914) and Bock (1928) for the many form which they studied. Blood capillaries are present in the goldfish only where the nervosa fibers penetrate the substance of the gland.

TABLE I.

Differential cell counts on the transitional lobe of the pituitary gland of the goldfish.

	Mean	S.E.	*Diff. of Means* / *S.E. of Diff.*	*Coeff. of variation*	*Number of pituitaries*
Acidophiles in Common	45	.54	9.2	3.1	7
Acidophiles in Moor	56	1.1		5.0	7
Basophiles in Common	55	.54	8.1	2.6	7
Basophiles in Moor	45	1.2		6.7	7

In relation to the presence of the blood vessels in this part of the gland in the goldfish, it is interesting to note the occurrence of masses of colloid material. Herring (1908) was the first to describe colloid in the gland and he believed it to be a secretory product from one of the lobes. More recently, Matthews (1936) reported large masses of this material among the fibers of the stalk in *Fundulus*. The presence of large blood vessels in the pars nervosa of the goldfish together with Florentin's (1934) concept of a hypophyseal portal system which drains the epithelial portions of the gland, through these vessels in the pars nervosa, indicates that if secretions are poured directly into the blood stream they probably find their way into the pars nervosa. Thus, the presence of colloid between the fibers of the pars nervosa may be the result of a concentration of some secretory product and appears to support some of the earlier concepts of pituitary secretion.

Another point worthy of note in regard to the pars nervosa is its relation to the pars intermedia. In most forms, thus far reported, the nervosa is connected directly to the intermedia without coming into contact with any other portion of the pituitary gland. In the two forms of the family Cyprinidae (carp and goldfish) which have been studied, the pars nervosa must first pass through the transitional lobe before it reaches the pars intermedia.

In the cod an interesting condition exists (Herring, 1908) which may be interpreted as being intermediate between the more common arrangement and the apparently special situation occurring in the carp and its related form, the goldfish. In the cod the intermediate lobe is divided into two parts by a chromophilic band of cells and one part is invaded directly by strands of the pars nervosa while, to reach the other portion of the intermedia, the strands of the nervosa must first go through the chromophilic band of tissue. It may very well be that this chromophilic band of tissue represents the transitional or übergangsteil portion of this gland. It should be noted in passing that recognition of that part of the pituitary did not occur until sometime after Herring's investigation (Stendell, 1913).

The cells of the intermediate lobe are described as small and taking a basic or acidic strain depending upon previous fixation. It was seen that if an osmic acid fixing solution is used the cells do not stain with acid fuchsin. Occasionally they retain some aniline blue which gives them a basophilic appearance. After the use of a non-osmic acid fixing solution these same cells stain red with acid fuchsin or eosin treatment. Gentes (1907), Herring (1908) and Matthews (1936) found that the cells in the intermediate lobe did not take any stain after the methods which they employed. Other workers, however, have reported acidophiles or basophiles and sometimes both to be present at one time. In the eel, Tilney (1911) and Stendell (1914) described small basophilic cells with clear cytoplasm. Stendell also found a few weakly staining eosinophiles present while Bock (1928) mentioned that the basophilic pars intermedia cells contained acidophilic granules in their cytoplasm. Matthews (1936) found not only acidophiles and basophiles in the intermediate lobe, but cells which took no stain and others which stained a pale lavender after azur carmine. As a result of these investigations, it can be seen that all types of cells have been reported in the intermediate lobe of the fish pituitary gland. The cells present may be of only one type or a mixture of several types. The fact that the affinity of the same cells for different stains can be modified by the type of fixing solution used has been overlooked by most workers. Uniformity in the types of fixing solutions employed is a necessary precaution before the results of different investigations can be compared.

In general, the Golgi network present in the cells of the pituitary gland of the goldfish appears to be characteristic for each epithelial lobe. In the intermediate lobe the Golgi network is present as a fine, heavily granulated structure capping the nucleus. Although the cells of the intermediate lobe are divided into granular and agranular types, the Golgi structure is the same in both forms. This may indicate that the cells are of the same kind and that the difference in granulation is due merely to phases of physiological activity. The same picture is seen in the acidophiles of the anterior lobe. The Golgi apparatus of the granular and non-granular cells, as well as that of the intermediate cell types, is exactly alike. There is a difference in the extent of blackening between the Golgi apparatus of the granular and agranular cells. On the basis of our present concept of the activity of the Golgi apparatus as related to cellular secretion, this change in stainability may be indicative of cellular activity.

The cells of the transitional lobe differ markedly among themselves in staining reaction, granulation, and in the form and position of the Golgi apparatus. The basophiles contain a Golgi network which is large, loosely arranged, free in the cytoplasm, and usually not in contact with the nucleus. The Golgi network of the acidophiles is compact, smaller, and caps the nucleus. The presence of two types of Golgi configurations in the chromophilic cells of the transitional lobe of the goldfish pituitary is a condition similar to that reported by Addison (1916), Atwell (1929) and Severinghaus (1933) for the anterior lobe of the mammal. Severinghaus (1933) has pointed out that the different Golgi structures present in the chromophilic cells can also be found in the chromophobic cells. This led him to state that the chromophobes could be divided into acidophilic chromophobes and basophilic chromophobes, suggesting that the acidophilic cells arise from the acidophilic and basophilic cells from the basophilic chromophobes. Kirkman (1937), in the guinea pig, also described two types of Golgi configurations in the chromophobes. Similarly in the present work, two types of Golgi configurations can be distinguished in the chromophobic cells of the transitional lobe. The presence of two types of Golgi networks in the chromophobes suggests (on the basis of the work of Severinghaus), that a relationship exists between these cells and the chromophilic cells of the transitional lobe. The compact type of Golgi network in the chromophobes resembles the same structure in the acidophiles while the loose, more strandlike type can be associated with the loosely arranged, ramifying Golgi network of the basophilic cells.

From the above discussion it can be seen that the Golgi apparatus of the anterior lobe cells in the goldfish pituitary is different from that found in the cells of the transitional lobe. This may be used as added evidence in support of the contention that the cells of the transitional lobe are not related to the cells of the anterior lobe in the goldfish. The great similarity between the Golgi configurations present in the transitional lobe cells of the goldfish and the anterior lobe cells of higher forms gives a firmer basis for postulating a homology between the two lobes.

Another item of interest in the present investigation is the comparison of the pituitary glands from the two different varieties of goldfish studied on the basis of the differential counts of the chromophilic cells in the transitional lobe. A statistical analysis of these cell counts shows that a significant difference does exist in the percentages of chromophilic cells present in the transitional lobes of these two varieties. Table I shows that in the forms investigated, there are more basophilic cells present in this lobe of the common variety than are present in the same lobe of the black moor variety. This difference in cell counts may possibly be associated with the morphological differences existing in the two forms of goldfish under observation. The black moor has been shown to contain a greater number of acidophiles. These cells in the anterior lobe of the mammal are believed to be associated with growth, and changes in their number from the normal are related to abnormal changes in their morphology (Crew, 1923; Smith & MacDowell, 1930; Stockard, 1931; Cushing, 1932, a,b; and Vicari, 1937). All these workers found, after investigating a form which showed marked differences, anatomically, from others of the same species, that these differences were associated with a change in the pituitary gland. They concluded that differences in body structure may be reflections of cellular differences in the pituitary. The abnormal growth of the black moor as well as the presence of telescopic eyes and black pigmentation may also, in some way, reflect the differences in cell counts occurring in their pituitary glands.

In the goldfish, as well as in other forms examined, in which morphological differences occur, these differences in structure are not necessarily restricted to the individual animal but may be transmitted to the offspring. That is, of course, if the form being studied can survive to sexual maturity. Stockard's investigations have indicated that the inheritance of these abnormal forms, which may owe their abnormality to some changed activity of a gland in the organism, follows a definite genetic ratio.

Goldfish breeders have found that when two of the so-called fancy types of goldfish are mated, the offspring may range in form from that of the common variety to forms even more divergent, morphologically, than the parents. Many of the young are so abnormal that they die during the first few days of life. Others appear to be the common variety for several weeks and then begin to show atypical form. If this phenomenon is dependent on the glands of internal secretion, it is at this time that the glands causing the change in body types would be most apt to show the greatest variation from that found in the typical variety which undergoes no radical morphological change. If the pituitary is really the gland which is modifying body type, then an examination of that gland in the two varieties at that possible "critical" period should yield greater divergence of cell type, distribution, or number, than reported as the result of the present investigation. It is planned to obtain the necessary materials to carry out an examination of pituitaries in animals sacrificed at the "critical" periods of development.

Summary and Conclusions.

1. Confirmation is offered for the anatomical position and relations of the goldfish pituitary gland and its morphological division into four lobes, the pars anterior, pars nervosa, pars intermedia and the transitional lobe.
2. The pars anterior contains acidophilic cells with a granular Golgi network applied to the nucleus.
3. The pars intermedia consists of both granular and agranular chromophobic cells each containing a similar granular Golgi apparatus which caps the nucleus.
4. The transitional lobe contains the following three types of cells:

   (a) A basophilic cell which contains large granules and an extensive loosely arranged Golgi network made up of fine strands and lying free in the cytoplasm.

   (b) An acidophilic cell which has a finely granular cytoplasm and contains a heavy strand Golgi network capping the nucleus.

   (c) Two varieties of chromophobes are present. Both have a scanty cytoplasm but can be differentiated by their Golgi configuration. One variety has a compact heavy Golgi apparatus while the Golgi apparatus of the other is looser and more strand-like. These cells are described as being related to the different chromophilic cells found in the transitional lobe of the gland in a way similar to that described by Severinghaus for the pars anterior of the mammal.
5. The pituitary glands of the two varieties of goldfish studied (the common and the black moor) are anatomically and histologically similar.
6. Statistical evaluation of cell counts made on the transitional lobe of both forms shows a significant difference in the proportion of chromophilic cells. The black moor contains a greater number of eosinophiles than does the common variety.
7. The morphological differences between the black moor and the common variety of goldfish may possibly be related to the differences in the proportional distribution of the different chromophilic cells in their pituitary glands.

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EXPLANATION OF THE PLATE.

Plate I.

Fig. 1. Section of the pars intermedia showing the relationships of the granular and agranular cells to each other and to the invading strands of the pars nervosa. × 240 Champy. Masson stain.

Fig. 2. A group of cells from the pars intermedia showing the compact Golgi apparatus capping their nuclei. × 1350 Nassonov-Kolatchev. Masson stain.

Fig. 3. A group of cells from the pars anterior showing the granular Golgi apparatus next to the nuclei. × 1350 Nassonov-Kolatchev. Masson stain.

Fig. 4. A group of transitional lobe cells showing the large granules in the basophilic cells and the highly granular, heavily staining acidophilic cells. × 450 Champy. Masson stain.

Fig. 5. A section showing the heavy strand, compact Golgi apparatus capping the acidophilic cells of the transitional lobe. × 1350 Nassonov-Kolatchev. Masson stain.

Fig. 6. A group of chromophobic cells from the transitional lobe showing the types of Golgi apparatus present in these cells. One type is more loosely arranged and made up of thinner strands than the other. × 1350. Nassonov-Kolatchev. Masson stain.

Fig. 7. A group of basophilic cells from the transitional lobe containing a loosely arranged strand-like Golgi net in the cytoplasm of the cells. × 1350 Nassonov-Kolatchev. Masson stain (Compare with Fig. 5).

Fig. 8. A group of cells from the pars anterior showing the complete lack of cellular arrangement present here. × 600 Champy. Masson stain.

Fig. 9. A section of the gland showing the ramifications of the pars nervosa and its intimate connection with the large blood vessels of the gland. × 170 Champy. Masson stain.